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REGULAR ARTICLE

Heat tolerance of Portuguese old bread wheat varieties

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Abstract

Wheat is a major staple crop, and its grain yield is affected by heat stress. Such environmental constraint frequently occurs in the main Portuguese wheat producing regions. The aim of this work was to evaluate the impact of high temperatures after anthesis on gas exchanges, chlorophyll a fluorescence, membrane integrity and yield in nine Portuguese old bread wheat varieties. Photosynthetic rate (Pn) reductions occurred in Gentil Rosso, Grécia and Nabão and may result from inactivation of PSII activity, as indicated by decreases in photochemical efficiency under light (Fv'/Fm') and in quantum yield of electron transport (ϕ_e). Results denoted an enhancement/maintenance of photosynthetic ability under heat, expressed by stable stomatal conductance (gs) and higher water use efficiency (WUE) in MEQ and Restauração, increased Pn in MEB and Restauração, and Pn stability in Ruivo. Reduced membrane damage (lower leakage) in Ruivo and MEQ suggested a higher protoplasmic tolerance to heat in these varieties. Control plants of MEQ also presented the highest lipid amount and the less unsaturated membrane lipids (low double bond index), and these traits were unaffected by heat. Ruivo denoted a stimulation of lipid biosynthesis which could have positive implications on thermal tolerance. Increased Pn and WUE, stable gs and abundant lipids in control plants (MEQ, T94, MEB, Restauração) corresponded to kernel yield increases under heat. Physiological traits are expected to contribute to Portuguese wheat breeding programs towards high temperature tolerance.

Key words: High temperature, Photosynthetic activity, Membrane integrity, *Triticum aestivum*, Yield

Introduction

Wheat is a staple food for more than 35% of the world population. It is also one of the main grain crops in Portugal. South Portugal (Alentejo) is an important wheat producing region, characterized by Mediterranean climate conditions, where increasing water stress associated with high temperatures become more frequent and reduce crops yield (Maçãs, 1996). Mean daily temperature of 15ºC is considered optimal for wheat development (Chowdhury and Wardlaw, 1978), but above this threshold a reduction in the duration of grain filling period is observed (Al-khatib and Paulsen, 1984). In Alentejo, mean daily temperatures above 15ºC, and maximal temperatures above 32ºC, are frequent during grain filling period (March to the end of June). Abiotic stresses such as drought and heat are predicted to occur more frequently due to climate change (Arnell, 1999). Wheat grain production depends on successful reproductive development (Wang et al., 2012), and the selection of adequate genotypes to face such environmental constraints may contribute to mitigate stress impact on productivity. Wheat genetic breeding programs in Alentejo have been concerned with heat tolerance traits among others (Maçãs, 1996; Barradas et al., 1996; Almeida, 2007).

Ecophysiological traits are useful to assess plant responses to environmental changes (Campos et al., 1999; Ramalho et al., 1999; Shvaleva et al., 2008; Matos et al., 2009). Under high temperature conditions, photosynthetic rates measured during grain filling have been positively associated with yield (Quinn and Williams, 1985; Reynolds, 1998; Erdei et al., 2002). High temperatures may damage plant cell membranes resulting in photosynthesis reductions. Heat stress induces the production of

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reactive oxygen species, which can bring about peroxidation of membrane lipids, leading to membrane injury (Blum and Ebercon, 1981; Almeselmani et al., 2006; Leshem, 1992), photosynthesis impairment and leaf senescence (Zhao et al., 2007). As observed for other stressful conditions, the ability to maintain membrane integrity and, hence, cell compartmentalization and metabolism, is crucial for plants survival under heat stress. That relies, mostly, on the dynamics of the lipid matrix of cellular membranes, which includes quantitative and qualitative modifications upon stress exposure (Matos et al., 2002; Dias et al., 2010; Scotti-Campos et al., 2011).

The aim of this work was to evaluate photosynthetic performance and membrane integrity in nine old Portuguese bread wheat varieties subjected to high temperatures after anthesis**,** under greenhouse controlled conditions. Evaluation was based on leaf gas exchanges, chlorophyll *a* fluorescence, membrane integrity and yield. Characterization of heat tolerance responses is expected to contribute to breeding programs aiming at a better adaptation of future wheat varieties to warm environments.

Materials and Methods

Seeds from 7 old Portuguese wheat varieties, were used in this study. These were: Gentil Rosso (G. Rosso), Grécia, Mocho de Espiga Branca (MEB), Mocho de Espiga Quadrada (MEQ), Restauração (Restaur.), Ruivo, Transmontano 94 (T94). These varieties were previously selected from a wheat germplasm (Vasconcelos 1933) belonging to INIAV. Two commercial varieties (Ardila, Nabão) were also sown in order to allow a comparison with currently used cultivars. Sowing took place in December in a greenhouse (INIAV/Oeiras) with controlled temperature conditions, in 7 L pots. These were filled with clay loam soil collected from the field (Lisbon), mixed with 20 g of granular fertilizer (Blaukorn, Bayer, N:P:K:micronutrients, 12:12:17:2). Four pots were used for each genotype, with a sowing density of 7 seeds per pot and 2.5 cm depth. Temperature, relative air humidity and radiation were monitored by means of two data loggers (Mezão Lda., Portugal). Diurnal maximal temperature and relative humidity varied between 23-26 ºC and 50-60 %, respectively, along the experimental period. Natural irradiation ranged from 400 to 1000 µmol m^{-2} s^{-1} along the day inside the greenhouse.

Fourteen days after sowing (DAS) germinated seeds were thinned to five plants per pot, tillers were removed along development and the plants were well watered throughout the growing period. Ten days after visual assessment of anthesis (during the grain filling period, 3.5 months old plants), which corresponded to different dates according to varieties, two pots of each genotype were left under control conditions while the two remaining pots were placed in a different greenhouse compartment. The heat stress treatment consisted in the plant exposure to maximal temperatures *ca*. 10 ºC higher than the control (reaching *ca*. 40 ºC, during 4 h each day, for 7 days). Watering was maintained in both compartments, to avoid RWC lowering of heat exposed plants. At the end of the high temperature imposition, measurements were performed in adult flag leaves and pots returned to the compartment under control conditions. Plants were allowed to continue until full maturation (*ca*. 150 DAS), and were individually harvested.

Leaf gas exchanges

The evaluation of net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (E) and water use efficiency (WUE) was performed during the morning period (10:00-12:00), using a portable CO_2/H_2O, infrared gas analyzer exchange system LI-6400 (LI-COR Inc., Lincoln, USA), with an external CO_2 concentration of *ca*. 370 µL L^{-1}, chamber block temperature controlled at 25ºC, and artificial light adjusted at 1000 µmol m^{-2} s^{-1} supplied by a "cold" lamp LED type. The parameters were calculated according to the equations of Caemmerer and Farquhar (1981). The sensor head encloses a leaf surface of up to 6 cm^2, well stirred to minimize boundary layer resistance as referred by Matos et al. (1998). For each parameter, the mean value of three measurements (minimum) is presented.

Chlorophyll a fluorescence

Diurnal Chl *a* fluorescence parameters were determined immediately after gas exchange measurements, under the same environmental conditions, using a PAM 2000 system (H. Walz, Effeltrich, Germany), as described in Ramalho et al. (2003), following the formulae reported elsewhere (Krause and Jahns, 2004; Schreiber, 2004). Briefly, the maximal PSII efficiency of energy conversion under light (F_v'/F_m'), the estimate of the quantum yield of non-cyclic electron transport (ϕ_e) and the photochemical quenching (q_P), which denotes the proportion of energy trapped by PSII and driven to photochemical events, were obtained under photosynthetic steady-state conditions, using a PPFD of ca. 1000 µmol m^{-2} s^{-1} of actinic light (provided by a halogen lamp from the PAM-2000)

and superimposed saturating flashes (of *ca.* 7000 µmol m^{-2} s^{-1}).

Electrolyte leakage

For each variety, four leaves (one per plant) were cut into 1 cm^2 sections. Pooled samples of 7 leaf sections (three replicates per treatment) were floated on 10 mL of deionized water for 22 h. Conductivity values resulting from electrolytes released by cells were read using a conductimeter Crison GLP 31 (Crison Instruments, Spain), at *ca.* 20ºC. Total conductivity was measured after sample exposure to 90ºC in an oven for 2 h, followed by cooling. Membrane leakage was expressed as a percentage of the total conductivity.

Lipid analysis

Lipid analysis was performed as earlier described in Campos et al. (2003). For each variety, four leaves (one per plant) were cut into 1 cm^2 sections, and pool samples (*ca.* 1 g FW, three replicates per treatment)) were boiled for 2 min in distilled water to stop lipolytic activities. Total lipids were extracted in a mixture of chloroform/methanol/water (1/1/1, by vol.) according to Allen et al. (1966). For fatty acids (FAs) analysis, aliquots of total lipids extract were saponified and methylated with BF_3-methanol (Merck), using heptadecanoic acid (C17:0) as internal standard (Metcalfe et al., 1966). Two methylation replicates were performed for each extract.

The fatty acid methyl esters were analysed with a gas-liquid chromatograph (Varian, CP-3380, USA) equipped with a flame-ionization detector. Separation was carried out on a capillary DB-Wax column (J & W Scientific, 0.25 mm i.d. x 30 m, 0.25 µm), as described in Campos et al. (2003). Column temperature was programmed to rise from 80ºC to 200ºC at a rate of 12ºC min^{-1}, after 2 min at the initial temperature. Injector and detector temperatures were 200°C and 250°C, respectively. Carrier gas was hydrogen with a flow rate of 1 ml min^{-1}, at a split ratio of 1:100 of the sample. Fatty acids were identified by comparison with known Sigma standards. The value for the total fatty acids (TFA) corresponds to the sum of individual FAs. The unsaturation degree of TFA was obtained through a double bond index (DBI), calculated according to the formula: DBI = [(%monoenes + 2 x %dienes + 3 x %trienes) / % saturated FAs] (Mazliak, 1983).

Grain yield

Plants were individually harvested at full maturity (*ca.* 150 DAS) and threshed manually after oven drying of the shoots at 35ºC for 72 h. Grain yield (kernel weight per plant) was determined.

Statistical analysis

A two-way ANOVA ($P<0.05$) was applied, using Statistix 9 (Analytical Software, 2009), followed by a Tukey test for mean comparison (95% confidence level).

Results

Plant water status and gas exchanges

As regards net photosynthesis (P_n), Nabão and MEB presented the highest and the lowest absolute values (14.5 and 9.2 µmol m^{-2} s^{-1}, respectively), under control conditions (Figure 1). Heat stress induced significant P_n decreases in MEQ, Gentil Rosso, Transmontano 94, Ardila, Grécia and Nabão (24%, 18%, 14%, 12%, 10% and 5%, respectively), whereas P_n remained unaltered in Ruivo, and increased in MEB (36%) and Restauração (8%).

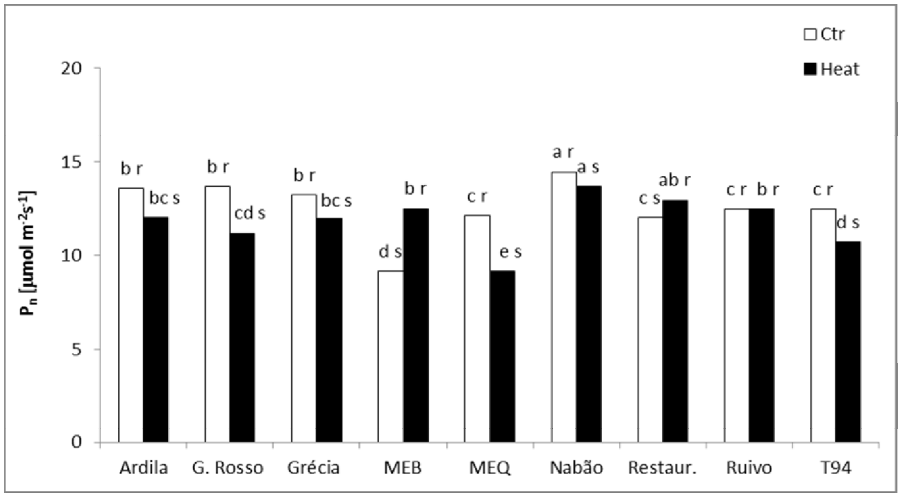

Figure 1. Changes in net photosynthesis (P_n) in leaves of nine *T. aestivum* genotypes, under control (Ctr) or heat conditions imposed after anthesis. Different letters express significant differences between genotypes for each treatment (a, b, c, d, e) or between control and heat treatment for the same genotype (r, s).

Ruivo presented the highest g_s in control plants, whereas the lowest value was found in MEB (136.3 µmmol m^{-2} s^{-1}). However, the latter presented the highest rise (165%), to 361.4 µmol m^{-2} s^{-1}, after heat exposure (Table 1). High temperature also induced significant g_s increases in Nabão (116%), Ardila (85%) and Grécia (61%), whereas the other genotypes maintained values close to their respective controls.

Accordingly to the highest g_s rise amongst genotypes, MEB presented also the highest transpiration (E) increase (346 %). Increases in E also occurred in all other genotypes (ranging from 207% in Grécia to 20 % in Ruivo) except MEQ and Restauração, what is in accordance with their stable g_s values. As regards internal CO_2 (C_i), a decrease was observed only in Restauração (26%). Small increases were observed in Ruivo (7%) and Gentil Rosso (4%). In all the others C_i increased (from 32 to 55%), being maximal raises observed for MEB (Table 1). Water use efficiency (WUE) increased in MEQ and Restauração (85% and 65%, respectively), whereas all the others genotypes showed WUE reductions (Table 1), particularly in the case of MEB and Nabão (68-70% decreases).

Chlorophyll a fluorescence

In control plants, Ardila and Ruivo showed the lowest PSII photochemical efficiency (F_v'/F_m') and photosynthetic electron transport (ϕ_e) values (Table 2). After high temperature imposition, a significant decrease of F_v'/F_m' occurred in Gentil Rosso (28%), Grécia (18%) and Nabão (12%). MEQ, Restauração, Ruivo and Transmontano 94 revealed only non-significant decreases, whereas Ardila and MEB showed to be completely unaffected. Quite similar variations were found for ϕ_e, namely in the reductions of 31%, 24% and 11% showed by Gentil Rosso, Grécia and Nabão, respectively. As regards the photochemical quenching (q_p), no differences were found between genotypes under control. Furthermore this parameter was unaffected by heat in all genotypes (Table 2), except in Grécia that presented a 6.5% drop.

Electrolyte leakage

Under control conditions, the lowest electrolyte leakage was obtained in Ardila, Nabão and Restauração, whereas Ruivo presented the highest membrane leakage (Figure 2). Heat stress did not promote an increase in membrane permeability in none of the genotypes, with significant reductions being observed in MEQ and Ruivo.

Table 1. Changes in stomatal conductance (g_s), transpiration (E), internal CO_2 concentration (C_i) and water use efficiency (WUE) in leaves of nine *T. aestivum* genotypes under control (Ctr) or heat conditions imposed after anthesis.

Genotype	Treatment	g_s mmol m^{-2} s^{-1}	E mmol m^{-2} s^{-1}	C_i µmol mol^{-1}	WUE P_n / E
Ardila	Ctr	176.9 $^{c\,s}$	3.4 $^{bc\,s}$	232.4 $^{c\,s}$	4.1 $^{c\,r}$
	Heat	326.7 $^{bc\,r}$	7.9 $^{b\,r}$	306.4 $^{b\,r}$	1.6 $^{d\,s}$
G. Rosso	Ctr	171.8 $^{c\,r}$	3.9 $^{a\,s}$	236.0 $^{c\,s}$	3.6 $^{de\,r}$
	Heat	184.4 $^{d\,r}$	6.7 $^{c\,r}$	244.9 $^{e\,r}$	1.7 $^{cd\,s}$
Grécia	Ctr	193.0 $^{bc\,s}$	3.3 $^{cd\,s}$	249.8 $^{b\,s}$	4.2 $^{c\,r}$
	Heat	310.6 $^{c\,r}$	10.0 $^{a\,r}$	301.8 $^{bc\,r}$	1.2 $^{d\,s}$
MEB	Ctr	136.3 $^{d\,s}$	1.5 $^{f\,s}$	186.7 $^{d\,s}$	6.0 $^{a\,r}$
	Heat	361.4 $^{b\,r}$	6.9 $^{c\,r}$	289.5 $^{bc\,r}$	1.9 $^{cd\,s}$
MEQ	Ctr	222.3 $^{b\,r}$	3.3 $^{bc\,r}$	188.8 $^{d\,s}$	3.7 $^{d\,s}$
	Heat	218.1 $^{d\,r}$	3.5 $^{d\,r}$	285.5 $^{c\,r}$	6.9 $^{a\,r}$
Nabão	Ctr	225.2 $^{b\,s}$	3.0 $^{de\,s}$	245.6 $^{bc\,s}$	6.3 $^{b\,r}$
	Heat	486.9 $^{a\,r}$	7.2 $^{bc\,r}$	330.8 $^{c\,r}$	1.9 $^{cd\,s}$
Restaur.	Ctr	187.9 $^{bc\,r}$	3.7 $^{ab\,r}$	254.9 $^{b\,r}$	3.5 $^{de\,s}$
	Heat	211.6 $^{d\,r}$	3.4 $^{e\,r}$	189.2 $^{f\,s}$	5.8 $^{b\,r}$
Ruivo	Ctr	303.6 $^{a\,r}$	3.8 $^{a\,s}$	281.2 $^{a\,s}$	3.5 $^{e\,r}$
	Heat	319.0 $^{c\,r}$	4.7 $^{d\,r}$	301 $^{bc\,r}$	2.7 $^{c\,s}$
T94	Ctr	220.1 $^{b\,r}$	2.9 $^{e\,s}$	188.1 $^{d\,s}$	4.2 $^{c\,r}$
	Heat	225.0 $^{d\,r}$	6.4 $^{c\,r}$	268 $^{d\,r}$	2.0 $^{cd\,s}$

Different letters express significant differences between genotypes for each treatment (a, b, c, d, e) or between control and heat treatment for the same genotype (r, s).

Table 2. Changes in photochemical efficiency of PSII under photosynthetic steady-state conditions (F_v'/F_m'), quantum yield of photosynthetic electron transport (ϕ_e) and photochemical quenching (q_p) in leaves of nine *T. aestivum* genotypes under control (Ctr) or heat conditions imposed after anthesis.

Genotype	Treatment	F_v'/F_m'	ϕ_e	q_p
Ardila	Ctr	0.394 $^{b\,r}$	0.351 $^{b\,r}$	0.891 $^{a\,r}$
	Heat	0.407 $^{a\,r}$	0.357 $^{a\,r}$	0.866 $^{a\,r}$
G. Rosso	Ctr	0.458 $^{ab\,r}$	0.414 $^{ab\,r}$	0.904 $^{a\,r}$
	Heat	0.328 $^{a\,s}$	0.284 $^{a\,s}$	0.866 $^{a\,r}$
Grécia	Ctr	0.486 $^{ab\,r}$	0.445 $^{ab\,r}$	0.916 $^{a\,r}$
	Heat	0.396 $^{a\,s}$	0.338 $^{a\,s}$	0.857 $^{a\,s}$
MEB	Ctr	0.420 $^{ab\,r}$	0.344 $^{ab\,r}$	0.823 $^{a\,r}$
	Heat	0.427 $^{a\,r}$	0.388 $^{a\,r}$	0.910 $^{a\,r}$
MEQ	Ctr	0.565 $^{a\,r}$	0.509 $^{a\,r}$	0.901 $^{a\,r}$
	Heat	0.516 $^{a\,r}$	0.478 $^{a\,r}$	0.927 $^{a\,r}$
Nabão	Ctr	0.487 $^{a\,r}$	0.432 $^{ab\,r}$	0.885 $^{a\,r}$
	Heat	0.430 $^{a\,s}$	0.383 $^{a\,s}$	0.890 $^{a\,r}$
Restaur.	Ctr	0.533 $^{a\,r}$	0.399 $^{ab\,r}$	0.746 $^{a\,r}$
	Heat	0.498 $^{a\,r}$	0.374 $^{a\,r}$	0.751 $^{a\,r}$
Ruivo	Ctr	0.335 $^{b\,r}$	0.290 $^{b\,r}$	0.859 $^{a\,r}$
	Heat	0.306 $^{a\,r}$	0.256 $^{a\,r}$	0.836 $^{a\,r}$
T94	Ctr	0.399 $^{ab\,r}$	0.337 $^{ab\,r}$	0.845 $^{a\,r}$
	Heat	0.330 $^{a\,r}$	0.299 $^{a\,r}$	0.904 $^{a\,r}$

Different letters express significant differences between genotypes for each treatment (a, b, c, d, e) or between control and heat treatment for the same genotype (r, s).

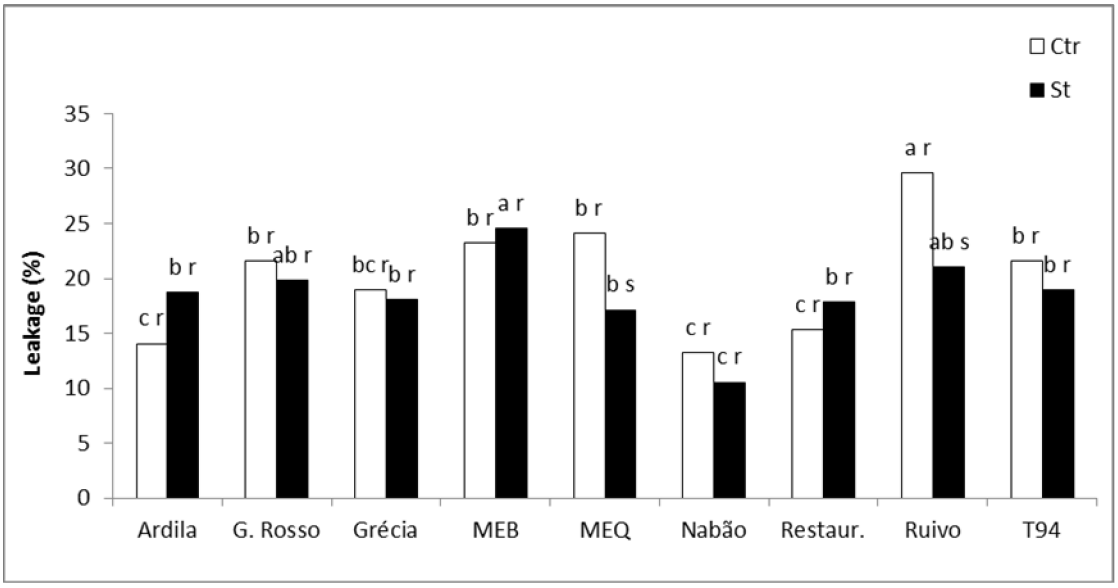

Figure 2. Changes in electrolyte leakage values in leaf sections of nine *T. aestivum* genotypes, under control (Ctr) or heat conditions imposed after anthesis.
Different letters express significant differences between genotypes for each treatment (a, b, c, d, e) or between control and heat treatment for the same genotype (r, s).

Lipid analysis

As regards total fatty acids (TFA), under control conditions (Table 3) the highest contents were observed in Transmontano 94 and MEQ (ca. 19-20 mg g^{-1} DW), followed by MEB (15 mg g^{-1} DW) and Restauração (13.5 mg g^{-1} DW). The lowest TFA content was found in Ruivo (5.2 mg g^{-1} DW), Ardila (7 mg g^{-1} DW) and Grécia (9 mg g^{-1} DW). After heat treatment no significant differences were found in lipid amounts except for Ruivo, where a 100% increase was observed (Table 3).

Concerning the individual fatty acids (FA), in control plants linolenic acid (C18:3) was by far the

most abundant FA, followed by palmitic (C16:0), linoleic (C18:2) and palmitoleic (C16:1) acids (Table 3). After high temperature exposure, plants presented a stable FA composition in relation to control. The only significant changes were found for T94 (decrease of C18:3 and increase of minor FA), Ruivo and MEB (lower and higher C16:0, respectively).

As regards TFA unsaturation degree, the heat treatment did not significantly influence the double bond index (DBI). However, lower DBI values occurred in MEQ and Transmontano 94, both under control and after high temperature exposure, indicating higher membrane FA saturation in these two cultivars (Table 3). In both the unsaturated palmitic acid (C16:0) was clearly more abundant (30-37%) than in the remaining cultivars (13-17%), regardless of treatment. Furthermore the highly unsaturated linolenic acid (C18:3) was found in lower percentages in MEQ, and decreased in Transmontano 94 after heat treatment.

Grain yield

High temperatures induced an increase (>10% in relation to control) in grain yield (kernel production per plant) in MEQ (38%), Nabão (18%), Transmontano 94 (18%), and MEB (12%) (Figure 3). Gentil Rosso and Restauração presented small positive variations, whereas the remaining genotypes showed negative yield impacts, with decreases of 13%, 20% and 21% in Grécia, Ruivo e Ardila, respectively.

Table 3. Changes in fatty acid composition (mol %), total fatty acids (TFA) content and unsaturation (DBI) of total lipids in leaves of nine *T. aestivum* genotypes under control (Ctr) or high temperature conditions imposed after anthesis. Different letters express significant differences between genotypes for each treatment (a, b, c, d, e) or between control and heat treatment for the same genotype (r, s).

		<16:0	16:0	16:1 *c+t*	18:0	18:1	18:2	18:3	TFA mg g^{-1}	DBI
		mol %							DW	
Ardila	Ctr	10.9 ^{br}	14.8 ^{cr}	4.7 ^{ar}	2.4 ^{ar}	1.8 ^{br}	9.6 ^{bcr}	55.8 ^{bcr}	7.1 ^{dr}	11.4 ^{abr}
	Heat	9.2 ^{cdr}	13.0 ^{br}	5.7 ^{ar}	2.8 ^{br}	2.3 ^{br}	8.1 ^{er}	58.9 ^{ar}	7.6 ^{er}	13.0 ^{ar}
G. Rosso	Ctr	6.7 ^{cr}	14.2 ^{cr}	2.6 ^{br}	1.4 ^{bcr}	1.4 ^{br}	10.5 ^{bcr}	63.3 ^{ar}	10.7 ^{cr}	13.9 ^{ar}
	Heat	938 ^{cr}	15.0 ^{br}	2.1 ^{cr}	1.3 ^{der}	2.3 ^{br}	11.5 ^{cdr}	57.9 ^{ar}	10.3 ^{dr}	12.7 ^{ar}
Grécia	Ctr	6.8 ^{cr}	13.5 ^{cr}	1.9 ^{br}	1.9 ^{br}	1.7 ^{br}	9.4 ^{cdr}	64.8 ^{ar}	8.7 ^{dr}	14.5 ^{ar}
	Heat	8.9 ^{cdr}	14.0 ^{br}	3.4 ^{br}	2.2 ^{bcr}	2.0 ^{bcr}	11.5 ^{cdr}	58.0 ^{ar}	10.4 ^{dr}	12.7 ^{ar}
MEB	Ctr	4.3 ^{cdr}	17.1 ^{cr}	2.0 ^{bs}	1.1 ^{bcr}	1.7 ^{br}	14.4 ^{ar}	59.5 ^{abr}	14.7 ^{br}	11.7 ^{abr}
	Heat	3.9 ^{er}	16.6 ^{br}	3.0 ^{cr}	0.9 ^{er}	1.4 ^{cr}	15.8 ^{ar}	58.3 ^{ar}	14.9 ^{br}	13.1 ^{ar}
MEQ	Ctr	12.5 ^{abr}	37.2 ^{ar}	2.4 ^{br}	1.9 ^{br}	1.7 ^{br}	15.0 ^{ar}	29.3 ^{dr}	19.2 ^{ar}	3.2 ^{cr}
	Heat	13.7 ^{br}	31.2 ^{ar}	2.7 ^{cr}	1.9 ^{cdr}	1.8 ^{bcr}	12.7 ^{bcr}	36.0 ^{cr}	19.5 ^{ar}	4.2 ^{br}
Nabão	Ctr	5.5 ^{cr}	14.3 ^{cr}	4.8 ^{ar}	1.3 ^{bcr}	1.4 ^{br}	13.3 ^{ar}	59.3 ^{ar}	10.9 ^{cr}	13.9 ^{ar}
	Heat	6.7 ^{der}	16.1 ^{br}	4.4 ^{br}	2.2 ^{bcr}	1.6 ^{bcr}	13.1 ^{abcr}	55.9 ^{abr}	12.7 ^{cr}	10.9 ^{ar}
Restaur.	Ctr	2.1 ^{dr}	16.1 ^{cr}	5.1 ^{ar}	1.0 ^{cr}	1.1 ^{br}	12.2 ^{abr}	62.3 ^{abr}	13.5 ^{br}	12.8 ^{abr}
	Heat	4.8 ^{er}	16.8 ^{br}	5.6 ^{ar}	2.0 ^{bcr}	1.7 ^{bcr}	15.1 ^{abr}	54.1 ^{abr}	11.6 ^{cdr}	10.8 ^{ar}
Ruivo	Ctr	15.1 ^{ar}	16.4 ^{cr}	4.3 ^{ar}	3.0 ^{ar}	2.7 ^{ar}	6.7 ^{ds}	51.7 ^{cr}	5.2 ^{es}	10.2 ^{br}
	Heat	16.5 ^{ar}	12.9 ^{br}	2.3 ^{cs}	3.7 ^{ar}	3.5 ^{ar}	10.6 ^{cder}	50.5 ^{br}	10.4 ^{dr}	11.0 ^{ar}
T94	Ctr	4.7 ^{cds}	30.3 ^{br}	3.0 ^{br}	1.0 ^{cr}	1.1 ^{br}	9.0 ^{cdr}	51.0 ^{cr}	20.3 ^{ar}	5.8 ^{cr}
	Heat	9.7 ^{cr}	35.6 ^{ar}	3.0 ^{cr}	1.6 ^{cdr}	1.5 ^{cr}	9.8 ^{der}	38.9 ^{cs}	17.9 ^{ar}	4.0 ^{br}

DBI=[(%monoenes + 2 x %dienes + 3 x %trienes) / %saturated FAs]

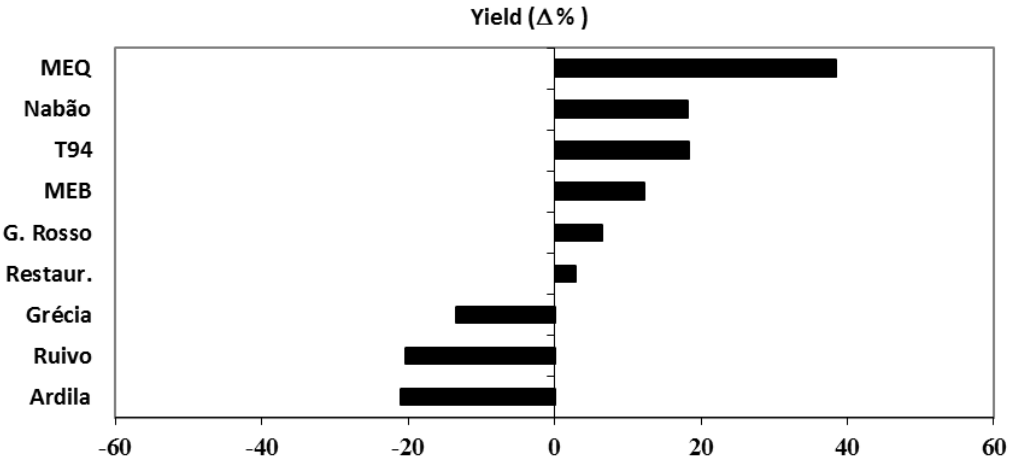

Figure 3. Changes induced by heat in grain yield of nine *T. aestivum* genotypes, expressed as percentage of variation of kernel yield (g plant^{-1}) in relation to control.

Discussion

The optimum temperature to achieve maximum yields of wheat is generally considered to be between 15 and 20ºC during grain growth (Dupont and Altenbach, 2003). However, short periods (3-5 days) of high temperature ($\geq$ 35ºC) occur quite often during the grain filling stage of wheat (Semenov and Halford, 2009), in the major Portuguese producing regions. It was reported that high temperature reduces the rate of transport of assimilates from vegetative organs to kernels, resulting in significant grain yield reductions, although high-temperature sensitivity differed amongst varieties (Plaut et al., 2004). Therefore, the present experimental conditions intended to study the high temperature impact in yield by simulating heat stress peaks that frequently occur in the field immediately after anthesis. Results concerning leaf gas exchanges denoted some variability in wheat responses to high temperature. Higher or stable P_n may occur, but the majority of genotypes depicted P_n decreases. It is conceivable that CO_2 gas-exchange is hindered at high temperatures by closing of the stomata (Matos et al., 1998).

Excessive heat increases transpiration and leaf water deficit may rapidly become so severe that stomata may close as a result of water-stress. In our case, the P_n decreases did not result from stomatal closure, since all genotypes showed higher or unaltered g_s values, accompanied by C_i increases, except in Restauração and Ruivo (which did not show P_n reductions). Therefore, when existing, the observed P_n drop would result from non-stomatal effects that hampers photosynthetic metabolism. As temperature increases, the CO_2 compensation point may rise (especially with C_3 plants), resulting in an increase in intercellular CO_2 concentration (Bauer et al., 1975). Also, the decline in net photosynthesis at high temperature in C_3-grasses is due to a strong increase in respiration (Matos et al., 1998).

Effectively the high temperature limit of net CO_2 uptake (maximum temperature for net photosynthesis or heat compensation point) is a resultant of two counteracting temperature-dependent processes. One involves the enzyme reactions of photosynthesis, the rates of which increases with rise in temperature in the lower and medium range, but are strongly inhibited by high temperatures. The other is respiration, which increases more rapidly than P_n with rising temperature (Bauer et al., 1975).

Considering sources for non-stomatal disturbances in P_n the impact on PSII activity might have contributed to P_n reductions in Gentil Rosso, Grécia and Nabão, as inferred from decreases of F_v'/F_m' and (ϕ_e), observed in these genotypes. High temperatures could induce hyperfluidization of thylakoid membranes, affecting lipid-protein interactions and causing various disturbances, such as phase transitions of lipids and conformational changes (Raison et al., 1982). The transition from liquid crystalline to liquid phase of bulk thylakoid lipids causes changes in microenvironment of chlorophylls (Tovuu et al., 2013), that may alter photochemical efficiency of PSII. The remaining genotypes displayed quite stable values of F_v'/F_m', (ϕ_e) and q_P, suggesting a lower thermal sensitivity of the photochemical apparatus of photosynthesis to heat (Matos et al., 2002).

On the other hand, photosynthetic activity was stimulated after heat exposure in MEB, whereas MEQ and Restauração displayed stable g_s and WUE enhancement, as well as greater kernel yield increases (g plant^{-1}) under heat, particularly in MEQ (38% increase). MEQ overcame P_n impairment (24% decrease) and Restauração slightly increased P_n (8%) and presented a concomitant and unique C_i reduction (26% decrease). In a former comparison of ancient bread wheat cultivars, the highest photosynthetic rates occurred in Restauração under heat stress (Scotti Campos et al., 2011a), what is in accordance with our present results which denote an enhancement of photosynthetic ability in these genotypes under high temperatures.

The maintenance of chlorophyll *a* fluorescence values in most of the genotypes further agrees with the impact on cell membranes. Differences in membrane selectivity and membrane injury have been widely used as an indicator of protoplasmic tolerance to abiotic stresses (Campos et al., 2003; Matos et al., 2009; Scotti-Campos et al., 2011a). Changes in membrane lipid composition may highlight plant mechanisms that help to avoid or to cope with membrane damage that would result in impaired cell metabolism (Campos et al., 2003; Partelli et al., 2011). Higher membrane stability (lower leakage) occurred in Ruivo and MEQ under heat. In the case of Ruivo, a higher protoplasmic tolerance as well as unaffected P_n may be related to the stimulation of membrane lipid biosynthesis, as inferred from higher TFA values under heat. This may correspond to a plant acclimatory response to assist membrane repair, eventually occurring under stress as reported for other species under environmental stressful conditions (Campos et al., 2003; Matos et al., 2009; Partelli et al., 2011; Scotti-Campos et al., 2011b; Medeira et al., 2012). In MEQ high membrane stability is in accordance with high lipid content (high TFA) and low unsaturation of membrane lipids (low DBI) in control plants, which were unaltered under heat. All genotypes showing high lipid amounts in control plants displayed raises in kernel yield, except Nabão. Previous studies showed that, under heat stress, genotypes MEQ and Ruivo displayed high P_n values while maintaining considerable yields, expressed as 1000-kernel weight (Scotti-Campos et al., 2011a). Present results further points that membrane lipid composition, as well as preservation of membrane integrity and stability, may also be involved in the maintenance of a better photosynthetic performance of several genotypes after exposure to high temperatures.

Conclusions

Results obtained in this work highlighted several responses among genotypes that can be related to a better performance under heat stress. Stable g_s and WUE enhancement in the cultivars MEQ and Restauração, increased P_n in the cultivars MEB and Restauração, or P_n stability in the cultivar Ruivo, denote an enhancement/maintenance of photosynthetic ability under high temperatures, in accordance with previous observations. Except for Nabão, more abundant lipids in control plants of cultivars MEQ, T94, MEB and Restauração corresponded to better kernel yield increases (g plant^{-1}) under heat. MEQ also presented the less unsaturated membrane lipids (low DBI) in control plants, which were unaltered under heat conditions. A stimulation of lipid biosynthesis was observed in Ruivo. Such traits related to membrane composition and stability in Ruivo and MEQ might explain their reduced membrane damage (lower leakage) under stress, suggesting a higher protoplasmic tolerance to heat.

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